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Letter to the Editor: Assignment of the ^{1}H, ^{13}C and ^{15}N resonances and secondary structure of the monomeric p13^{suc1} protein of *Saccharomyces pombe*

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Biological context

The cyclin dependent kinases (CDK) drive the progression of the mitosis. In addition to the activating cyclin protein, the kinase complex contains a small subunit called CKS for cyclin-dependent kinase subunit (Brizuela et al., 1987). CKS proteins are essential for the entry and exit of mitosis and target the wee1 and CDC25 kinase regulators to phosphorylation by the CDKs (Patra et al., 1999). The CKS protein from *Saccharomyces pombe*, p13^{suc1}, exists both in a closed conformation (Endicott et al., 1995) and in a C-terminal β-strand exchanged conformation (Bourne et al., 1995; Khazanovich et al., 1996). Mutation of the hinge proline P90 of p13^{suc1} from *S. pombe* to alanine has been previously shown to stabilize the protein and to prevent swapping to occur (Rousseau et al., 1998). We have started NMR studies of the CKS protein from *Saccharomyces pombe*, p13^{suc1} and the PA90 mutant. We have shown by NMR chemical shift perturbations that the p13^{suc1} of *S. pombe* binds via its conserved anion-binding site to a CDC25 phosphatase peptide in a phosphorylation-dependent way (Landrieu, 2001; Odaert, 2002). We present here the backbone assignment of the wild type and the complete assignment for the PA90 mutant protein.

Methods and experiments

Expression and purification were described previously (Odaert et al., 2002). [^{15}N] and [^{15}N, ^{13}C]-labeled wild-type and p13PA90 mutant proteins were expressed in the minimal medium M9 supplemented by [^{15}N] ammonium chloride (1 g l^{-1}) and [^{13}C] glucose (2 g l^{-1}) (Cambridge Isotopes Laboratories, Cambridge, MA). NMR samples contained 1–2 mM labeled protein in 100 mM NaCl 50 mM Na_2HPO_4/NaH_2PO_4 (pH 6.8) in 5%:95% D_2O/H_2O or 100% D_2O.

NMR data were recorded at 20 °C on a Bruker DMX600 (Pasteur Lille) and on a Varian Inova 600 (GBB) equipped with a triple-resonance 5 mm probe with a *z* gradient coil. Data were processed on an O2 workstation with the program SNARF v0.8.9 (Frans van Hoesel, University of Groningen). Backbone assignment was achieved with the 3D triple resonance experiments (Sattler et al., 1999): HNCA/HN(CO)CA and HNCO/HN(CA)CO. Sidechain resonance assignment was achieved with the following experiments: HNCACB, CBCA(CO)NH, HBHA(CBCACO)NH, (CO)N(CO)CAH (Dijkstra et al., 1997), HCCH-TOCSY,^{15}N-edited TOCSY-HSQC and ^{13}C-HSQC (aromatic region). The assignment was confirmed and completed with ^{15}N-edited NOESY-HSQC (100 ms mixing time) and ^{13}C-edited HSQC-NOESY (36 ms mixing time) in H_2O and D_2O.

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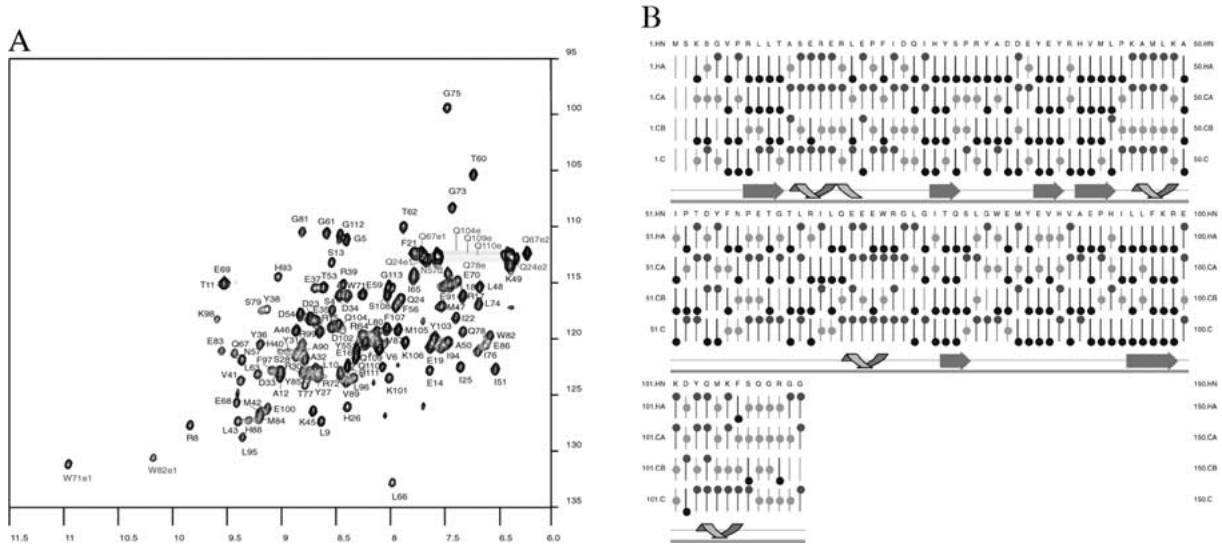

Figure 1. (A) Annotated ^{1}H-^{15}N HSQC spectrum of the mutant PA90 protein at 293 K and a ^{1}H resonance frequency of 600 MHz. The assignment of the peaks is indicated with the residue number. (B) Secondary structure in solution of the p13^{suc1} protein, derived from the consensus between the chemical shift indexes (CSI) for the ^{13}CO, $^{13}C\alpha$, $^{13}C\beta$ and $^{1}H\alpha$ resonances of the PA90 mutant protein (Wishart et al., 1994).

Extent of assignments and data deposition

Only a partial backbone assignment ($^{13}C\alpha$, $^{13}C\beta$, ^{13}CO, ^{15}N) was achieved for the wild-type protein, due to a linebroadening of the resonances in the C-terminal β-sheet (residues 84–97). Mutation of the hinge proline P90 into an alanine suppressed this linebroadening, but had no effect on the protein fold, as supported by only subtle differences in the chemical shifts. Sidechain assignment was therefore performed on the p13^{suc1} PA90 mutant protein. A complete assignment was achieved, except for residues M1, S2 and R39, for the ^{13}C resonances of the aromatic rings (H, Y, F, W) and of the acidic groups (D, E), and for the resonances of the sidechain amide groups (K, R). Resonances for the residues involved in the anion binding site showed partial (R30, S79, W82 and R99) or extreme (R39) linebroadening, depending on the phosphate concentration (Landrieu, 2001). The chemical shifts for the wild-type and for the PA90 mutant protein have been deposited in the BioMagResBank under the accession numbers BMRB-5008 and BMRB-5009 respectively.

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